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Rapid generation of activated mononuclear antigen presenting cells from monocytes

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RAPID GENERATION OF ACTIVATED MONONUCLEAR ANTIGEN PRESENTING CELLS FROM MONOCYTES

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The present invention relates to new activated mononuclear antigen-presenting cells, means processes and kit for preparing the same, and their use for the preparation of pharmaceutical compounds.

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Dendritic cells (DCs) are professional antigen-presenting cells, derived from hematopoietic progenitor cells, playing a central role in the initiation of the immune response to pathogens (1-3). The recent progress on DC biology has revealed the heterogeneity of these cells and various types of DCs endowed with different phenotypic and functional properties have been characterized under both *in vivo* and *in vitro* conditions (4). However, the mechanisms regulating generation, functions and survival of DCs in response to infections or exposure to foreign or modified antigens are largely unknown.

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Upon exposure to pathogens and other danger signals, DCs undergo a complex maturation process, characterized by the expression of a distinct pattern of membrane molecules, which parallels the acquisition of high competence for antigen presentation and migration to the lymphoid organs (3). One typical marker of DC maturation is CD83, a 45,000 Mr glycoprotein belonging to the Ig superfamily, predominantly expressed *in vivo* on the surface of some DC cell subsets, including skin Langerhans cells and interdigitating reticulum cells present in the T cell zones of lymphoid organs (5). Although the physiological function of CD83 is still unknown, an association with the generation of highly active DCs is presumed, as it may play an important role in Ag presentation and/or cellular interactions (6).

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Monocytes are highly versatile cells playing crucial roles in the maintenance of immune homeostasis. These cells circulate in the bloodstream, transmigrate through vascular endothelium and localize in peripheral tissues, where they mature into different types of tissue histiocytes or other cell types. *In vitro* studies have shown that monocytes can be the precursors of a particular subset of myeloid DCs (generally named as DC1), whose generation requires several days of culture in the presence of cytokines, followed by a further incubation step necessary to induce DC maturation (2, 4). In particular, even though some authors reported the appearance of a variable

percentage of CD83⁺ DCs after a single step treatment of monocytes, either after transmigration across an endothelial barrier (7) or after a 3 day exposure to GM-CSF and type I interferon (8), it is generally thought that the expression of CD83 on monocyte-derived DCs requires two sequential events: i) the induction of DC differentiation from monocytes, after exposure for several days to GM-CSF and IL-4 (9); ii) induction of maturation, characterized by CD83 expression, after the subsequent addition of stimuli such as LPS, CD40L, virus infection or other factors (2). Although this method for preparing monocyte-derived DCs has allowed to carry out important studies on the biology of DCs, the physiological relevance of this pattern of DC differentiation is questionable, especially because exposure of monocytes to IL-4 can hardly mimic the cytokine milieu likely to be present at the infection site.

Distinct subtypes of human circulating DCs have been detected in the blood. In particular, Zhou and Tedder had described a rare population of human blood DCs selectively expressing CD83 (10). Moreover, circulating CD2⁺ monocytes have been considered as DC precursors (11) and as a special subset of peripheral blood DCs (12). However, no relationship was established between CD2 and CD83 expression and freshly isolated CD2⁺ monocytes were described as CD83-negative cells (12). CD83⁺ cells represent a very low percentage (< 1%) of circulating mononuclear cells.

The inventors report the unexpected finding that membrane CD83 expression is induced, by post-transcriptional mechanisms, in CD2⁺/CD14⁺ monocytes soon after cell seeding, concomitantly with the acquisition of marked DC activities. IFN- α , but not IL-4, favors the persistence of membrane CD83 expression during culture time and rapidly renders these cells highly competent for inducing a primary T cell response.

Advantages of the invention are given in that it describes a new cell population containing functional activated mononuclear antigen presenting cells (APCs). Said population is obtainable thereby from freshly isolated PBMCs or monocytes after a single step treatment. The invention also provides a particularly rapid procedure for the production of these new APCs. This procedure may furthermore be carried out without any *ex vivo* culture step, in a close single use system, therefore avoiding possible contaminations and limiting manipulations and transports.

The present invention relates to a cell population prepared from blood mononuclear cells, said mononuclear cells being peripheral blood mononuclear cells (PBMC) or CD14⁺ monocytes, said population containing from about 10 % to about 50

% of activated mononuclear antigen presenting cells having the following characteristics:

- they express surface markers CD2, CD83 and CD14, MHC class I and MHC class II molecules
- they secrete TNF- α
- they are able to stimulate allogenic T lymphocytes, as shown by MLR measurements
- they are able to stimulate autologous T lymphocytes proliferation in an antigen specific way.

Mononuclear cells particularly preferred for the preparation of cells according to the invention are possibly total unseparated peripheral blood mononuclear cells (PBMC), isolated from blood by any process known by a person skilled in the art, such as apheresis. Total PBMCs contain mainly monocytes, lymphocytes and Natural Killer (NK) cells. As an alternative, monocytes may be isolated from blood, for example by density gradient centrifugation, adherence or positive immunoselection using anti-CD14 conjugated magnetic beads. These monocytes isolated from blood are designated as CD14⁺ monocytes. An important fraction of this CD14⁺ monocytes population also expresses CD2 marker on its surface and are then designated as CD14⁺/CD2⁺ monocytes.

By "antigen-presenting cells" (APCs) is meant cells able to present antigens to T lymphocytes and to stimulate their proliferation. By "activated mononuclear APCs" are defined mononuclear cells characterized by the membranar or extracellular expression of proteins which were not synthesised or not exported by non-activated mononuclear cells, but which corresponding mRNA were already present in non-activated mononuclear cells, therefore allowing protein expression within a few hours process. Activated mononuclear APCs also exhibit immunostimulating properties not presented by mononuclear cells. However, activated mononuclear APCs do not exhibit phenotypic or functional characters that are associated to cells differentiated from monocytes after several days of culture, such as the presence of dendrites, for monocyte-derived dendritic cells, or the expression of all co-stimulatory molecules, such as CD80. An other difference between activated mononuclear cells and differentiated monocytes resides in the high proportion of cells positive for the CD14 surface marker for the first ones.

Activated mononuclear APCs from a cell population according to the invention are better stimulators of T and B cell response than freshly purified monocytes. Therefore, such a cell population is suitable for the generation of an immune response in a patient in need thereof. Also, activated mononuclear APCs exhibit an immunophenotype (including receptors for monokines and chemokines), morphology, and immunological activity not present in dendritic cells (DCs), and particularly not in monocyte-derived DCs differentiated according to processes well known in the art (1, 2, 3).

If necessary, activated mononuclear APCs may be isolated from the cell population according to the invention by means such as elutriation and/or negative selection from lymphocytes on magnetic beads, for example on magnetic beads coated with anti-CD3 antibodies.

The present invention also relates to activated mononuclear APCs prepared from blood mononuclear cells and having the following characteristics:

- they express surface markers CD2, CD83 and CD14, MHC class I and MHC class II molecules
- they secrete more than 50 pg/ml of TNF- α
- they are able to stimulate allogenic T lymphocytes, as shown by MLR measurements
- they are able to stimulate autologous T lymphocytes proliferation in an antigen specific way.

In a particular embodiment, activated mononuclear APCs according to the invention do not secrete IL-10 and secrete less than 100 pg/ml of IL-12. In this respect, they differ from dendritic cells that may secrete high levels of IL12p70.

In an other particular embodiment, activated mononuclear APCs according to the invention are loaded with antigenic peptides or proteins, with a cellular extract containing at least one antigen or with nucleic acid molecules. Antigens against which the stimulation of an immune response is of interest are, for example, tumor-associated antigens, such as Melan-MART or MAGE, antigens overexpressed on tumors, such as Her-2/neu, MUC-1 or p53, or viral tumorigenic antigens, originating for example from Hepatitis C Virus, Human Papilloma Virus or Human T Lymphotropic Virus. They also can be infectious antigens from viral agents such as Human Immunodeficiency Virus,

Hepatitis B Virus, Hepatitis C Virus, or other viruses. A cellular extract may be composed with extracts from one or several different types or lineages of cells.

The present invention also relates to the use of cytokine having receptors on the surface of blood monocytes, of inducers of interferon synthesis by blood mononuclear cells or of a physical stress, as means allowing the preparation from blood mononuclear cells in an appropriate medium and for one to five hours, and preferably for less than four hours, of a cell population or of activated mononuclear APCs such as above described, said means being applied to the mononuclear cells from the initial stage of the preparation.

According to a particular embodiment of the invention, said cytokine having receptors on the surface of blood monocytes are chosen among a group consisting of: type I interferon (IFN), IFN-gamma, IL-12, IL-13, IL-18 and Granulocyte/Monocyte-Colony Stimulating Factor (GM-CSF). According to a more particular embodiment of the invention, said type I IFN is selected from the group consisting of any natural IFN α , any recombinant species of IFN α , natural or recombinant IFN β and any synthetic type I IFN. In a further embodiment of the invention, said type I IFN concentration in the medium is in a range of about 100 to about 100.000 IU/ml, and preferably in a range of about 1.000 to about 10.000 IU/ml.

According to a particular embodiment of the invention, said cytokine is used at a concentration in the medium ranging from about 0,01 to about 10 μ g/ml and preferably from about 0,1 to about 1 μ g/ml.

Means such as above described may be used alone or in combination. In a preferred embodiment of the invention, type-I Interferon or IL-13 are used in combination with GM-CSF. In a preferred embodiment, type I IFN is used at a concentration of about 10.000 IU/ml, in combination with GM-CSF at a concentration of about 500 IU/ml.

The present invention also relates to the use of a physical stress as means for the generation, of activated mononuclear APCs. Said physical stress may be chosen among a group consisting of one of the following events: the separation of blood mononuclear cells from the plasma contained in the blood initially containing the mononuclear cells, the exposure of the blood mononuclear cells to a temperature variation of +/- 3 to 8°C from 37°C, to an osmotic change or to an electrical field.

Another aspect of the present invention relates to a process for preparing, from blood mononuclear cells, a cell population or activated mononuclear APCs such as

above described, comprising a step of contacting said mononuclear cells with an appropriate medium for one to five hours and preferably for less than 4 hours.

By "medium" is meant any medium appropriate for the cells survival or culture and differing from blood. Any medium suitable for *ex vivo* culturing of mononuclear cells is suitable. However, in the specific case of further use of the cells for human patients, culture media like AIM-V (Gibco-Life Technologies) or X-VIVO 20 are preferably used.

In a particular embodiment, the process of the invention is characterized in that said medium contains, from the initial stage of the preparation, a component selected from the group consisting of: cytokine having receptors on the surface of blood monocytes and inducers of interferon synthesis by blood mononuclear cells.

By "initial stage of the preparation" is defined the moment where mononuclear cells are put in contact with the medium.

By "cytokine having receptors on the surface of blood monocytes" is meant any cytokine binding with high affinity to membrane receptors of monocytes and having transactivating properties. Said definition includes cytokines such as IL-12, IL-13, IL-18, as well as interferons (IFN), such as type I IFN or IFN-gamma. It also includes cell-growth factors, such as GM-CSF.

According to a particular embodiment of the invention, said cytokine is type I IFN and is selected from the group consisting of any natural IFN α , any recombinant species of IFN α , natural or recombinant IFN β and any synthetic type I IFN. In a further embodiment of the invention, said type I IFN concentration in the medium is in a range of about 100 to about 100.000 IU/ml, and preferably in a range of about 1.000 to about 10.000 IU/ml.

Using the latter range and concentration, activated mononuclear APCs acquire the optimal expression of membrane markers associated with functional activity, with minimal toxic effects and good cell viability.

In another particular embodiment, said cytokine is chosen among the group consisting of: IL-12, IL-13, IL-18 or GM-CSF, said cytokines being present in the medium at a concentration ranging from about 0,01 to about 10 μ g/ml and preferably from about 0,1 to about 1 μ g/ml.

The concentration of GM-CSF, which promotes monocyte survival in culture, is generally expressed as IU/ml. In a process according to the invention, it is preferably from about 100 to about 2000 IU/ml, and is preferably of about 500 IU/ml. About the

equivalence between IU and SI units, for GM-CSF "Leucomax" (Novartis), $1.67 \cdot 10^6$ IU correspond to 150 µg of product.

In another particular embodiment, interferon or cytokines, such as above described, may be used in combination. In a preferred embodiment, the medium contains type I IFN, at a concentration of about 10.000 IU/ml, and GM-CSF, at a concentration of about 500 IU/ml.

According to another embodiment of the invention, a process for preparing a cell population or activated mononuclear APCs such as above described may comprise a step of exposing said blood mononuclear cells to a physical stress, which can be: the separation of blood mononuclear cells from the plasma contained in the blood initially containing the mononuclear cells, the exposure of the cells to a temperature variation of ± 3 to 8°C from 37°C , to an osmotic change or to an electrical field. The "intensity" of such physical stress applied will be chosen so as to preserve viability and functionality of the mononuclear cells.

According to another embodiment, a process of the invention may be carried out by adding the medium with antigenic peptides or proteins, with a cellular extract containing at least one antigen or with nucleic acid molecules preferably encoding for antigens against which it is desirable to induce an immune response. Said added components are intended to be loaded onto the mononuclear cells. Cells may be loaded by direct adsorption of peptides on MHC molecules expressed on the surface of the cells, by phagocytosis of particulate antigens, pinocytosis of soluble antigens, affinity binding, fusion, intracellular nucleic acid (DNA or RNA) transfer, receptor mediated uptake or viral transfection according to methods known by a person skilled in the art.

The expression "inducers of interferon synthesis by blood mononuclear cells" relates to agents such as double stranded nucleic acids, to viral extracts, or to any other agent having such property known by a person skilled in the art.

In a further preferred embodiment, the process of the invention comprises:

- a first step of contacting mononuclear cells with an appropriate medium for about one to about five hours and preferably for less than about four hours, for the preparation of activated mononuclear APCs
- a second step of contacting said activated mononuclear APCs with a maturation agent.

Such an embodiment can be particularly suitable in all the cases, which can be identified by a skilled person, wherein a further maturation of the APCs obtained from

the mononuclear cells as reported above is desired. By "maturation agent" is meant any means known as to induce the maturation of dendritic cells resulting into phenotypic and functional modification of the cells (13). Said agent may, for example, been chosen among bacterial membrane extracts, such as poly IC, IFN gamma, CD40 ligand, or anti-CD40 antibody, peptides or proteins, such as OmpA (14), or other agents such as the clinically approved Broncho-Vaxom oral vaccine (15).

Accordingly, a process for the preparation of activated mononuclear APCs comprises a step of washing said activated mononuclear APCs, so as to eliminate possible residues of agents used for the preparation or the maturation of the cells. If activated mononuclear APCs of the invention are prepared directly from an apheresis product and in connection with an apheresis machine, washings may be easily carried out by said apheresis machine.

The invention also relates to activated mononuclear antigen presenting cells such as obtained by a process such as above described. The present invention also relates to activated mononuclear APCs kept under a frozen form, in an appropriate cryopreservative solution until reinjection to a patient. A part of the activated mononuclear APCs of the invention can be taken from the close system in which they are prepared and kept frozen, so as to be used for a later injection. Cells may be diluted in an appropriate medium, such as one containing 10 % of autologous serum + 10 % of dimethylsulfoxyde in a phosphate buffer saline. Cells according to the invention may also be conserved in a dehydrated form.

The invention also relates to a cell population or activated mononuclear APCs such as above described, or a composition comprising these cells, for use as a medicament.

The present invention further relates to a kit for preparing activated mononuclear APCs from blood mononuclear cells, in a close system allowing the exclusion of any conventional *ex-vivo* culture step, and comprising at least:

- -single use elements necessary for the activation process and the washings, including bag(s), appropriate medium, buffers and connecting tube(s), including connecting tube(s) to the apheresis machine.
- possibly a composition comprising type I IFN and compatible additives,
- possibly a composition comprising a cytokine and compatible additives,

- possibly a composition comprising a cell growth factor and compatible additives,
- possibly antigens to which an immune response is of interest

This kit contains means for the reduction to practice of the process described in the present application. A skilled person can easily identify the additives suitable in the compositions reported above.

A kit according to the invention may also possibly include:

- means for recovering and centrifuging blood to obtain a leucocyte concentrate,
- means for the recovery of mononuclear cells from PBMC;
- means for recovering lymphocytes and monocytes from other white cells and contaminating red cells,
- means for the transfection of cells and for targeting antigens to blood mononuclear cells or to the activated mononuclear-APCs.
- means for cell conservation, including for example glycerol or dimethylsulfoxide

A description of the invention is also given with the help of the annexed figure 7.

A further object of the present invention is a pharmaceutical composition or a vaccine comprising, as active principle, activated mononuclear APCs such as described above, together with a pharmaceutically acceptable carrier vehicle or an auxiliary agent, in a amount of about 10^5 to about 10^{10} , and preferably about 10^7 to about 10^8 of said cells per dose administered.

A further object of the present invention is a pharmaceutical composition or a cellular vaccine containing, as an adjuvant of an active principle, activated mononuclear APCs such as above described, in a amount of about 10^5 to about 10^{10} , and preferably about 10^7 to about 10^8 of said cells per dose administered.

Due to the properties of activated mononuclear APCs according to the invention and depending on the component they are associated with, said cells may be used as an active principle or as an adjuvant. Any vehicle, carrier, auxiliary agent and formulation adopted in art for manufacturing vaccines can be used in the vaccine of the invention. A skilled person can identify said components and all the steps of the relevant process of manufacturing.

The present invention also relates to the use of a cell population or of activated mononuclear APCs such as above described for the manufacture of a medicament for

treating an infectious or neoplastic disease. In a particular embodiment, said pathology is a tumor-associated disease.

5 The present invention also relates to a method for prophylaxis and/or therapy of pathologies associated with the presence of an antigen in the human body and comprising a step of administering a vaccine including an immunogen for said antigen and activated mononuclear APCs of the invention to a subject in need thereof. Such an antigen, including viral, bacterial and tumor antigens such as above described, can be any molecule which presence is associated with a pathology. The present invention particularly relates to a method for prophylaxis and/or therapy of neoplastic and of
10 infectious pathologies. Particular indications for neoplastic diseases are melanoma, prostate cancer, bladder cancer, breast cancer, blood malignancies and colorectal cancer. Anti-infectious pathologies particularly concerned are caused by Human Immunodeficiency virus, Hepatitis B and Hepatitis C virus.

15 The activated mononuclear APCs of the invention can be injected with specific antigens into a subject in need thereof, so that antigens are locally acquired by activated mononuclear APCs. The administration of activated mononuclear APCs of the invention is preferably intravenous in the peripheral circulation, at the end of the apheresis process, the patients possibly remaining connected to the machine during the whole process, or can also be directed at the site of the infection or within the primary
20 tumor, metastases or draining lymph nodes.

25 In a particular embodiment, blood mononuclear cells are collected from patients previously mobilized, by the administration of GM-CSF or Flt-3 ligand, so as to increase the amount and proportion of myeloid, monocytes and precursor cells in peripheral blood. This allows to perform a shorter collection of mononuclear cells, because of the processing of a smaller volume of treated blood and to focus the treatment on the ex vivo activation process.

30 The process according to the invention, using a close system for antigenic activation of APCs without any transfer of the cells to dedicated clean rooms and incubators, is of particular interest for patients with viral pathologies, such as HIV, HBV, HCV. These patients are usually not included in adoptive therapies for regulatory and security reasons. The process and kit of the invention allow the activation of the mononuclear cells and the loading of the antigens for which the induction of an immune response is of therapeutic interest, during the apheresis process, ensuring optimal safety and efficacy.

Legends of the figures and table

Figures 1A and 1B: *In vitro* up-regulation of CD83 expression on the cell membrane of freshly isolated CD14⁺ monocytes: correlation with the CD2 expression.

Figure 1A shows flow cytometric analysis showing CD83 and CD2 expression in freshly isolated monocytes ("0 hours", up and low left panels) *versus* monocytes cultured for 4 h in medium alone ("4 hours", up and low right panels). The left and right upper panels show the surface staining for CD83-PE (y axis) *versus* CD14-FITC (x axis), whereas left and right lower panels show the surface staining CD83-PE (y axis) and anti-CD2-FITC (x axis). Isotype control antibodies were used to set negativity limits according to their location. Similar profiles were obtained with monocytes from 10 different donors.

Figure 1B puts in evidence the linear relationship existing between the percentage of starting CD2⁺/CD14⁺ monocytes (x-axis) and the percentage of monocyte-derived CD83⁺ cells (y-axis) recovered after 4 h of culture. The correlation was evaluated by regression analysis (the regression coefficient is 0.633) using data obtained with monocytes from 18 donors.

Figures 2A, 2B and 2C: Post-transcriptional up-regulation of CD83 expression on the cell surface of cultured monocytes and correlation with acquisition of a high MLR activity.

Figure 2A shows the CD83 expression of isolated monocytes cultured in medium alone ("medium", up and left panel) or in medium containing actinomycin D 100 ng/ml ("ActD", up and right), cyclohexamide 100 μ M (Chx; low and left) or brefeldin A 5 μ g/ml (BfA; low and right) for 4 h. The dotted line indicates the FACS profile of cells stained with isotype control mAb. The data are representative of three independent experiments.

Figure 2B represents the RT-PCR analysis of CD83 mRNA in freshly purified monocytes (time 0) and monocyte-derived cells harvested at 4 h of culture. Negative control (CTR-) is represented by RNA extracted from a T cell line (CEM).

Allogenic mixed lymphocyte reaction (MLR) results are exhibited in figure 2C, with proliferation, in counts per minute (y axis), expressed in function of the stimulator/responder ratio (x axis). Freshly separated CD14⁺ cells (●) or 4 h-monocyte-derived cells (■) were cultured with allogeneic CD4⁺ T lymphocytes. Proliferation of control lymphocytes was lower than 1,000 cpm. The results shown are the mean \pm SE of values from triplicate wells and are representative of three independent experiments. Differences observed between CD14⁺ monocytes and monocyte-derived cells are statistically significant ($P < 0.04$).

Figures 3A and 3B: Immunophenotypic analysis of monocytes at early times after treatment with IFN- α /GM-CSF or IL-4/GM-CSF.

Phenotype of cells recovered after treatment of freshly isolated monocytes with either IFN- α /GM-CSF (Figure 3A) or IL-4/GM-CSF (Figure 3B) for 4 or 24 h. Dotted histograms represent cells stained with isotype-matched control mAbs. The upper and medium panels of each figure show the expression of CD2 and CD83 on cells recovered after 4 h (left) and 24 h (right) of treatment, respectively. The results from one representative experiment out of three are shown. The lower histograms of each figure show the expression of co-stimulatory molecules on cells recovered after 4 or 24 h treatment with either IFN- α /GM-CSF or IL-4/GM-CSF. The costimulatory molecule levels are expressed as mean of fluorescence intensity (MFI) \pm SE of values detected in three independent experiments. The numbers on the bars indicate the percentage \pm SE of cells expressing each antigen.

Figures 4A and 4B: Secretion of cytokines and expression of mRNA for type I IFN and IFN-induced genes in freshly isolated and cultured monocytes.

Supernatants from monocytes cultured for 4 h in medium alone or medium containing either IFN/GM-CSF or IL-4/GM-CSF were analyzed for the amounts of the different cytokines (Figure 4A). Shown is one representative experiment of 3 with monocytes from different donors. Differences in TNF- α levels of untreated and IFN/GM-CSF-treated cultures *versus* IL-4/GM-CSF-cultures are statistically significant ($P < 0.04$).

Figure 4B shows the RT-PCR analysis of mRNA expression for IFN- α , IFN- β , 2'-5'-AS and PKR in purified monocytes and in 4h-cultured cells, with medium alone, or monocytes cultured for 4 h in medium alone or medium. The data are representative of one out of three experiments.

Figures 5A and 5B: Antigen presentation ability of freshly isolated monocytes and monocytes cultured with or without cytokines and phenotypical maturation/activation of monocyte-derived cells following co-cultivation with autologous CD4⁺ T lymphocytes.

Freshly isolated monocytes or monocytes cultured for 4 h with or without cytokines were pulsed with inactivated-HIV-1 and then incubated with autologous CD4⁺ T lymphocyte. Figure 5A shows T lymphocytes proliferation, expressed as thymidine incorporation (y axis) relative to different stimulator/responder ratio (x axis). Proliferation of control CD4⁺ T lymphocytes was lower than 1,000 cpm. The results shown are the mean \pm SE of values from triplicate wells and are representative of three independent experiments. Differences observed between IFN/GM-CSF-treated cultures and IL-4/GM-CSF-treated cultures are statistically significant ($P \leq 0.05$).

Figure 5B shows phenotypical evolution of cells following co-cultivation with autologous CD4⁺ T lymphocytes.

Monocytes cultured for 4 h with or without cytokines (IFN or IL-4), incubated with autologous CD4⁺ T lymphocytes at a stimulator/responder ratio 1:5. After 4 days, the cells were recovered and analyzed by flow cytometry. Results are expressed as MFI, calculated by subtracting the MFI values of cultures stained with the isotype control mAb from the values obtained by staining with the specific mAbs. The results are representative of one out of three experiments.

Figure 6: In vitro up-regulation of CD83 expression on the cell membrane of APCs prepared from total PBMCs.

Left and up panel shows the flow cytometric analysis of the cell population obtained after 4h of exposition of total PBMCs to IFN and GM-CSF. The surrounded points correspond to the selected sub-population analysed for surface expression of the following surface markers: CD2, CD14, CD83. The up and right panel shows the cells

surface staining for CD2, the left and low panel shows the staining for CD14 and the right and low panel shows the CD83 staining.

Figure 7: Schematic representation of a kit according to the invention.

The graphic represents a conventional apheresis machine for which the ring concentrating blood mononuclear cells is linked to an additional non adherent plastic bag (Ethylene Vinyl Acetate or Teflon) previously filled with 50 to 500 ml of medium containing cytokine(s) or inducers of interferon synthesis and possibly the antigens to be loaded. After one to five hours of contact, the cells can either be injected directly to the patient's blood stream, or returned for centrifugation to concentrate and/or wash the activated mononuclear cells before re-infusion in autologous plasma to the patient.

In a possible setting, the plastic bag is connected to an electropulsation chamber, allowing the delivery of electric pulsations to the cell flow, and therefore stressing the mononuclear cells. A single use kit of bags and tubings is provided, allowing sterile connection to the apheresis machine.

1 designates the "cell activation bag" containing medium, cytokines/inducers of interferon synthesis and possibly antigens. 2 designates an optional flow electropulsation chamber. 3 represents a connection for control and possible additions, 4 represents the blood entry, 5 the mononuclear cells entering the cell activation bag. 6, 7 and 8 correspond, respectively, to the flow of activated mononuclear cells, red blood cells and plasma returning to peripheral blood.

Table 1: Cell surface molecules expressed by monocytes upon exposure to IFN/GM-CSF for 4 hours.

Relatively to the different surface markers (left column of the table), surface expression is indicated as the percentage of cells positive for the marker (middle column) and Mean Fluorescence Intensity (MFI, right column), directly related to the mean intensity of fluorescent signal associated to the cells and to the number of said molecules on the cell surface. MFI are arbitrary units, defined as relative fluorescence intensities for each fluorochrome in a cell sample (Becton Dickinson Immunocytometry Systems, Computer Based Training, vol. I, "Flow cytometry and Immunology basics).

Table 1: Cell surface molecules expressed by peripheral blood monocytes upon exposure to IFN/GM-CSF for 4 hours.

Marker	Percentage \pm S.D.	MFI \pm S.D.
CD2	50 \pm 23	20 \pm 8
CD83	49 \pm 26	24 \pm 12
CD14	96 \pm 3	223 \pm 24
CD1a	0	0
CD62L	7 \pm 5	25 \pm 15
CD4	88 \pm 3	38 \pm 5
CD40	7 \pm 6	14 \pm 10
CD80	0	0
CD86	98 \pm 2	49 \pm 18
CD33	96 \pm 2	130 \pm 10
CD25	0	0
LFA-1	95 \pm 1	1309 \pm 59
CD32	99 \pm 1	147 \pm 18
CD54	969 \pm 1	131 \pm 30
CD58	94 \pm 3	130 \pm 8
HLA-A,B,C	97 \pm 3	158 \pm 20
HLA-DR	98 \pm 2	600 \pm 108
ManR	3 \pm 1	21 \pm 1

5

Abbreviations used:

2'-5'-AS, 2'-5'-oligoadenylate-synthetase

10

APC: antigen presenting cell

AT, aldrithiol

DC, dendritic cell

FITC, Fluorescein IsoThioCyanate

GM-CSF: Granulocyte Monocyte Colony Stimulating Factor.

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ManR: Mannose receptor

PE, PhycoErythrin

PBMC: Peripheral Blood Mononuclear Cells

PKR, double-stranded-RNA-dependent protein kinase;

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Examples

Example 1: Induction of CD83 expression on the cell membrane of freshly isolated CD14⁺ monocytes after a short-term culture: correlation with the CD2 expression and with the acquisition of a potent MLR activity.

1) Monocyte-derived cell cultures

Monocytes were isolated from heparinized blood of normal donors by Ficoll and Percoll density gradient centrifugation (Seromed, Berlin, Germany) and positive immunoselection using anti-CD14 conjugated magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany). Monocytes were plated at the concentration of 2×10^6 cells/ml in RPMI 1640 (Gibco BRL, Gaithersburg, MD) supplemented with 10% FCS (Hyclone Laboratories, Logan, UT), (with or without the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF; 500 U/ml) and either IL-4 (1,000 U/ml) (R & D Systems, Minneapolis, MN) or IFN- α (10,000 U/ml, Alfaferone, Alfa-Wasserman, Italy) for 4-24 h). All reagents were tested for the absence of detectable levels of LPS, by Limulus Amebocyte lysate assay (Bio-Whittaker, New Jersey, USA).

2) Immunophenotypic analysis

Cells were washed and resuspended in PBS containing 1% human serum and incubated with fluorochrome-conjugated mAbs for 30 min at 4°C. The following mAbs were used for immunofluorescent staining: anti-CD14, CD80, (Becton Dickinson, San Jose CA), CD40, CD86, CD83, (Pharmingen, San Diego, CA), CD2 (clone SFCI3Pt2H9; Beckman Coulter, Fullerton, CA). Samples were collected and analysed by using a FACSort (Becton Dickinson) and data analysis was performed by CellQuest software (Becton Dickinson). Cells were electronically gated according to light scatter properties, in order to exclude cell debris and contaminating lymphocytes.

3) Statistical analysis

The correlation between the percentage of starting CD2⁺/CD14⁺ monocytes and the percentage of monocyte-derived CD83⁺ cells recovered after 4 h of culture was

analyzed by regression analysis. Statistical analysis was performed using unpaired Student's *t*-test.

4) Results

Circulating CD14⁺ monocytes were purified and cultured in medium without cytokine addition for 4 h; then, the loosely adherent cells were analyzed for the expression of CD83, CD14 and CD2 antigens with respect to freshly purified monocytes. While freshly isolated monocytes did not express CD83, a considerable percentage of the non adherent cells proved to be CD83⁺ following to their culture (range in 18 donors: 9-93%; median: 42.3%). Notably, these CD83⁺ cells were also positive for CD14 and CD2 antigens (Fig.1A). These monocyte-derived CD83⁺/CD14⁺/CD2⁺ cells expressed CD11c, CD13, CD33, CD45RO, CD86 and high levels of major histocompatibility complex class I and class II molecules, while were negative for CD1a and CD62L antigens (data not shown). In our study with monocytes from 18 donors, the percentage of CD2⁺ monocytes ranged from 4.5% to 50% of the total CD14⁺ circulating monocytes (median: 9%). There was a correlation ($r=0.79$, $P<0.005$ by regression analysis) between the percentage of monocyte-derived CD83⁺ cells after 4 h of culture and the percentage of CD2⁺/CD14⁺ cells at the onset of culture (Fig.1B), suggesting that CD2⁺/CD14⁺ monocytes are the immediate precursors of CD83⁺ cells. More complete data related to activated mononuclear APCs are summarized in table 1.

The rapid generation of monocyte-derived CD83⁺ cells suggests that CD83 surface expression could be regulated by post-transcriptional events. The CD83 surface expression in cells cultured in the presence of various metabolic inhibitors added at the beginning of the culture was examined. The up-regulation of CD83 expression on the cell membrane occurring after 4 h of monocyte culture was not significantly inhibited by treatment with the transcriptional inhibitor actinomycin D, whereas it was completely blocked by cyclohexamide or brefeldin A (Fig.2A), which inhibit protein translation and transport through the Golgi complex, respectively. These results indicated that CD83 expression was not due to "de novo" RNA transcription and that both synthesis and export of the CD83 protein on the cell membrane occurred within a short period of culture. Consistently with these results, remarkable levels of CD83 mRNA were expressed in purified monocytes as well as in cells recovered at 4 h of

culture (Fig. 2B). Intracellular immunostaining of cells recovered at 2 h of culture revealed the accumulation of the CD83 protein (data not shown). Of interest, the loosely adherent monocyte-derived cells recovered at 4 h of culture proved to be highly active APCs when tested in a typical MLR assay using allogeneic lymphocytes, while, as expected, monocytes poorly stimulated lymphocyte proliferation (Fig. 2C). Notably, when purified CD2⁺ monocytes were used as starting cell population, more than 90% of the cells recovered at 4 h were CD83⁺ and showed a high MLR activity (not shown). These results demonstrate that a particular type of CD83⁺/CD2⁺/CD14⁺ cells, highly active as APCs in MLR assays, is generated as early as 4 h after *ex vivo* incubation of freshly isolated CD14⁺ monocytes.

Example 2: Persistence of CD83⁺/CD2⁺ positive cells, secretion of cytokines associated with DC maturation and expression of IFN-induced genes

1) Cytokine detection

TNF- α , IL-12 and IL-1 β production was measured in the supernatants of monocyte-derived cells by commercial ELISAs (R&D Systems, Minneapolis, MN). ELISAs were performed in triplicate.

2) Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The mRNA from monocyte-derived cultures was extracted by RNAzol B (Tel-Test Inc.) and processed as previously described (13). Samples were amplified with specific primer pairs for CD83 (14), IFN- α , amplifying all 14 known human IFN- α subtype genes, and IFN- β as reported elsewhere (15). Primer sequences for IFN-inducible genes (2',5'-AS and PKR) were amplified as described elsewhere (16). β -actin RT-PCR was run in parallel to normalize the levels of human RNA in all the samples. All RT-PCR products were in the linear range of amplification.

3) Results

When monocytes were cultured in medium alone for further periods of times (24-48 h), CD83 and CD2 expression was progressively lost along with a decreased cell viability (data not shown). In order to investigate whether cytokines could play a role in the rapid generation/persistence of monocyte-derived CD83⁺ cells, purified monocytes were cultured with either IFN- α or IL-4, the cytokine typically used for generating immature DC from monocytes, in an experimental setting where GM-CSF was constantly included as a monocyte survival factor (Fig. 3).

At 4 h, CD83 was equally expressed in cultures treated with either IFN- α or IL-4 (similarly to the untreated cultures), while the CD2 levels were down-regulated in IL-4-treated cells (Fig. 3). At 24 h, no CD2 and CD83 expression was observed in IL-4-treated cultures, whereas IFN-treated cells were still positive for both markers (Fig. 3). The expression of costimulatory molecules was barely detectable (in the case of CD40 and CD86) or undetectable (CD80) in both types of cultures at 4 h. At 24 h, IFN-treated cells expressed higher levels of CD40 and CD80 with respect to the IL-4-treated counterparts (Fig. 3, panels A and B, bottom graphs).

To further characterize the differences observed among different monocyte-derived cells, the levels of TNF- α , IL-1 β and IL-12, pro-inflammatory cytokines known to be associated with DC maturation/activation, were measured in cell supernatants harvested at early time of culture (Fig. 4A). Detectable levels of these cytokines were revealed in all the cultures. However, a marked difference in the extent of TNF- α secretion was observed. In fact, at 4 h, both IFN-treated and untreated cultures secreted higher levels of TNF- α than IL-4-treated cells (Fig. 4A). At 24 h, there was a general decrease in the TNF- α levels (especially for untreated cultures); however, IFN-treated cells still exhibited a 3-fold increase in cytokine secretion as compared to the other cultures (data not shown). At both 4 and 24 h, IL-10 secretion was not detected in any cell culture (data not shown).

It was of interest to evaluate the expression of type I IFN genes as well as of genes typically up-regulated by IFN in freshly isolated monocytes with respect to cells cultured for a short time in the presence or absence of cytokines. Considerable levels of mRNA for both IFN- α and IFN- β were detected in freshly isolated monocytes as well as in monocyte-derived cells harvested at 4 h (Fig. 4B). Likewise, there was a clear-cut

expression of mRNA of the two IFN-induced genes 2'-5'-oligoadenylate-synthetase (2'-5'-AS) and double-stranded-RNA-dependent protein kinase (PKR) in both freshly isolated monocytes and 4 h-cultured monocyte-derived cells. Notably, the expression of both these IFN-induced genes was lower in IL-4-treated cells (Fig. 4B).

Example 3: A short-term exposure of monocytes to IFN- α results in the rapid generation of highly active APCs capable of inducing a primary T cell response.

1) Mixed lymphocyte reaction.

CD4⁺ T lymphocytes were sorted using anti-CD4 conjugated magnetic microbeads (Miltenyi Biotech) and seeded into 96 wells plates (Costar, Cambridge, MA) at 10⁵ cells/well. Allogeneic monocyte-derived cells were added (at a different ratio) to each well in triplicate. After 5 days, 1 μ Ci of methyl-³H-Thymidine (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was added to each well and incubation was continued for additional 18 h. Cells were finally collected by a Mach II Mcell (Tomtec, Amdem, CT) harvester and thymidine uptake was quantitated by liquid scintillation counting on 1205 Betaplate (Pharmacia, New Jersey, USA).

2) Primary response to HIV-1 antigens

CD4⁺ lymphocytes were purified and seeded into 96 wells plates (Costar) at 10⁵ cells/well. HIV-1 SF162 strain was inactivated by aldrithiol (AT)-2 treatment, following the procedure described elsewhere (17) and stored at -140°C until use. Loosely adherent cells recovered at 4 h of monocyte cultures were pulsed with AT-2-inactivated HIV-1 (2.4 ng of p24) for 30 min at 37°C and added to each well in triplicate, at a different ratios. After 7 days, 1 μ Ci of methyl-³H-Thymidine (Amersham) was added to each well for additional 18 hs. Cells were collected and thymidine uptake was quantitated as described above. For immunophenotypical analysis, monocyte-derived cell-T lymphocyte co-cultures were plated at ratio of 1:5. After 4 days, cells were collected and immunostained for FACS analysis. Analysis was performed by gating on monocyte-derived cells on the basis of the forward and side scatter properties.

3) Results

The phenotypic changes described above were correlated with the possible antigen presenting cell activity, as evaluated by a typical MLR assay using allogeneic lymphocytes as well as by measuring the capability of these cells to induce a primary antigen-specific autologous T cell proliferation. At 4 h of culture, untreated cells as well as cells treated with either IFN/GM-CSF or IL-4/GM-CSF showed a comparable potent MLR activity; at 24 h, however, IFN-treated cells proved to be better stimulators than both untreated and IL-4/GM-CSF-treated cells in MLR (data not shown). Of interest, when the different cell cultures were tested for their capability to stimulate the proliferation of autologous naive T cells after pulsing with the chemically inactivated HIV-1, a clear-cut difference in their functional activity was observed (Fig. 5A). In fact, at 4 h of culture, IFN-treated cells were more powerful stimulators than cells treated with IL-4/GM-CSF in inducing autologous CD4⁺ T cell proliferation at all the stimulator/responder ratios ($P \leq 0.05$). Similar differences between IFN-treated cultures and IL-4-treated cells were observed at 24 h (not shown). Freshly isolated monocytes as well as untreated cultures did not show any significant capability to stimulate a primary T cell response (Fig. 5A). Notably, when the 4 h-IFN-treated cells were co-cultured with autologous CD4⁺ T cells, a marked up-regulation of the expression of CD80, CD86, CD40 and CD83 was observed at 4 days. The expression of these markers was considerably lower in cultures treated with IL-4 or left untreated (Fig. 5B). Collectively, these results indicate that a short-term treatment of monocytes with type I IFN was sufficient to induce mature/activated APCs capable of efficiently inducing a primary T cell response.

Example 4: Induction of CD83 expression on the cell membrane of total PBMCs after a short-term culture.

Total mononuclear cells were collected by apheresis and suspended at 2×10^6 cells/ml in AIMV medium containing natural IFN α (10,000 IU/ml) and GM-CSF 500 IU/ml. All reagents were tested for the absence of detectable levels of LPS, by Limulus Amoebocyte lysate assay (Bio-Whittaker, New Jersey, USA).

After 4 hours of contact, the phenotype was studied as described in example 1. Cells were washed and resuspended in PBS containing 1% human serum and incubated with fluorochrome-conjugated anti-CD2 (clone SFC13P12H9; Beckman Coulter, Fullerton), anti-CD14, anti-CD83 (Pharmingen, San Diego, CA) mAb for 30 min at 4°C (Figure 6). Samples were collected and analysed by using a FACSort (Becton Dickinson) and data analysis was performed by CellQuest software (Becton Dickinson). Cells were electronically gated according to light scatter properties, in order to exclude cell debris and contaminating lymphocytes.

Cells were analyzed for the expression of CD2, CD14 and CD83 with respect to freshly purified monocytes.

Cells are seeded for MLR reaction as described in example 1, activated mononuclear APCs stimulate more efficiently allogenic T lymphocytes than do freshly isolated PBMCs or CD14+ monocytes.

Example 5 . Treatment of patients with chronic Hepatitis B or/and C

Patients with infectious diseases affecting peripheral blood cells (HIV, hepatitis...) are usually excluded from adoptive cellular therapies for safety and regulatory reasons.

In the present intention, those patients are treated during apheresis in a close system, by activating their blood peripheral immune cells in the presence of viral antigens and stimulating cytokines. These mononuclear cells are exposed to IFN α (1000 IU/ml) + GM-CSF (500 IU/ml), HCV antigenic epitopes isolated from virus capsids, HBS and HBC antigens at 1 μ g/ml in 250 ml RPMI medium for 4 hours. At the end of this contact in non adherent EVA plastic bags connected to the aphaeresis machine, the cells are transferred back in the core of the machine, centrifuged and resuspended in 50ml patients plasma for re-infusion. The patients return home after the 4 hours. aphaeresis session which is repeated after 2 weeks and again after 3 months.

Evaluation of this clinical protocol is conducted at months 6. What is observed is a negatvation of viral (HBV and/or HCV) load measured by PCR.

Example 6 : Treatment of patients with metastatic breast cancer

Patients with metastatic breast cancer after secure ablative surgery of primary tumor received combined chemotherapy and their mononuclear cells were mobilized by injection of GM-CSF.

These patients were then submitted to therapeutic apheresis. The mononuclear cells collected were transferred to a non adherent bag containing AIMV medium IL-18 1µg/ml plus IL-12 1µg/ml and oligopolylys -polyhistDNA plasmid coding for HER2/neu and for MUC-1 antigens. After 3 hours of contact, cells were submitted to a short electropulsation stress. They were kept for one hour at room temperature before washing by centrifugation in the apheresis machine, then re-suspended in 50 ml patients plasma and reinfused to the patient. This treatment is repeated after 1 month. The patients status is monitored by jetscan and clinical evaluation at months 3 and 6.

A progressive decrease in metastatic nodules is seen in lymph nodes and in bones.

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CLAIMS

1. A cell population prepared from blood mononuclear cells, said mononuclear cells being peripheral blood mononuclear cells (PBMC) or CD14+ monocytes, said population containing from about 10 % to about 50 % of activated mononuclear antigen presenting cells (APCs) having the following characteristics:

- they express surface markers CD2, CD83 and CD14, MHC class I and MHC class II molecules
- they secrete TNF- α
- they are able to stimulate allogenic T lymphocytes, as shown by MLR measurements
- they are able to stimulate autologous T lymphocytes proliferation in an antigen specific way.

2. Activated mononuclear APCs prepared from blood mononuclear cells and having the following characteristics:

- they express surface markers CD2, CD83 and CD14, MHC class I and MHC class II molecules
- they secrete more than 50 pg/ml of TNF- α
- they are able to stimulate allogenic T lymphocytes, as shown by MLR measurements
- they are able to stimulate autologous T lymphocytes proliferation in an antigen specific way.

3. Activated mononuclear APCs according to claim 2 characterized in that they do not secrete IL-10 and secrete less than 100 pg/ml of IL-12.

4. Activated mononuclear APCs according to claim 2 or 3, said activated mononuclear APCs having been loaded with antigenic peptides or proteins, with a cellular extract containing at least one antigen or with nucleic acid molecules.

5. Use of a cytokine having receptors on the surface of blood monocytes, of inducers of interferon synthesis by blood mononuclear cells or of a physical stress, as

means allowing the preparation from blood mononuclear cells in an appropriate medium, for about one to about five hours and preferably for less than about four hours, of a cell population according to claim 1 or of activated mononuclear APCs according to any one of claims 2 to 4, said means being applied to the mononuclear cells from the initial stage of the preparation.

6. A use according to claim 5 wherein said cytokine is type I IFN and is selected from the group consisting of any natural IFN α , any recombinant species of IFN α , natural or recombinant IFN β and any synthetic type I IFN.

7. A use according to claim 6, wherein said type I IFN concentration in the medium is in a range of about 100 to about 100.000 IU/ml, and preferably in a range of about 1.000 to about 10.000 IU/ml.

8. A use according to claim 5 wherein said cytokine is chosen among the group consisting of: IFN gamma, IL-12, IL-13, IL-18 and GM-CSF.

9. A use of a cytokine according to claim 5 or 8, wherein said cytokine concentration in the medium is in a range of about 0,01 to about 10 μ g/ml and preferably in a range of about 0,1 to about 1 μ g/ml.

10. A use according to claim 5, wherein said cytokines are used in combination.

11. A use according to claim 10 wherein said medium contains type-I interferon, at a concentration of about 10.000 IU/ml, and GM-CSF, at a concentration of about 500 IU/ml.

12. A use according to claim 5, wherein said physical stress consists of one of the following events: the separation of blood mononuclear cells from the plasma contained in the blood initially containing the mononuclear cells, the exposure of the blood mononuclear cells to an osmotic change, to an electrical field or to a temperature variation of +/- 3 to 8°C from 37°C

13. A process for preparing, from blood mononuclear cells, a cell population according to claim 1 or activated mononuclear APCs according to any one of claims 2 to 4, comprising a step of contacting said mononuclear cells with an appropriate medium, for about one to five hours and preferably for less than about four hours.

14. A process according to claim 13 characterized in that said medium contains, from the initial stage of the preparation, a component selected from the group consisting of: cytokine having receptors on the surface of monocytes, inducers of interferon synthesis by blood mononuclear cells

15. A process according to claim 14 wherein said cytokine is type I IFN and is selected from the group consisting of: any natural IFN α , any recombinant species of IFN α , natural or recombinant IFN β and any synthetic type I IFN.

16. A process according to claim 15, wherein said concentration of type I IFN in the medium is in a range of about 100 to about 100.000 IU/ml, and preferably in a range of about 1.000 to about 10.000 IU/ml.

17. A process according to claim 14 wherein said cytokine is chosen among the group consisting of: IFN gamma, IL-12, IL-13, IL-18 and GM-CSF.

18. A process according to claim 17, wherein said concentration of cytokine in the medium is in a range of about 0,01 to about 10 μ g/ml and preferably in a range of about 0,1 to about 1 μ g/ml.

19. A process according to claim 14, wherein said cytokines are used in combination.

20. A process according to claim 19, wherein said medium contains type-I interferon, at a concentration of about 10.000 IU/ml, and GM-CSF, at a concentration of about 500 IU/ml.

21. A process for preparing, from blood mononuclear cells, a cell population according to claim 1 or activated mononuclear APCs according to any one of claims 2

to 4, comprising a step of exposing said blood mononuclear cells to a physical stress, which can be: the separation of blood mononuclear cells from the plasma contained in the blood initially containing the mononuclear cells, the exposure of the cells to an osmotic change, to an electrical field or to a temperature variation of +/- 3 to 8°C from 37°C.

22. A process according to any one of claims 13 to 21, wherein said medium also contains antigenic peptides or proteins, a cellular extract containing at least one antigen or nucleic acid molecules.

23. A process according to any one of claims 13 to 22, wherein said process comprises:

- a first step of contacting mononuclear cells with an appropriate medium for about one to about five hours and preferably for less than about four hours, for the preparation of activated mononuclear APCs
- a second step of contacting said activated mononuclear APCs with a maturation agent.

24. Activated mononuclear APCs such as obtained by a process according to any one of claims 13 to 23.

25. A cell population according to claim 1 or activated mononuclear APCs according to any one of claims 2 to 4 or 24 wherein activated mononuclear APCs are kept under a frozen form, in an appropriate cryo-preservative solution

26. A cell population according to claim 1 or 25 or activated mononuclear APCs according to any one of claims 2 to 4 or 24 for use as a medicament

27. A composition comprising a cell population according to claim 1 or 25 or activated mononuclear APCs according to any one of claims 2 to 4 or 24 for use as a medicament

28. A kit for preparing, from blood mononuclear cells, a cell population according to claim 1 or activated mononuclear APCs according to any one of claims 2

to 4 or 24 activated mononuclear APCs, in a close system allowing the exclusion of any conventional ex-vivo culture step and comprising at least:

- single use elements necessary for the culture and the washings of the cells, including bag(s), culture medium, buffers and connecting tube(s), including connecting tube(s) to an apheresis machine.
- possibly a composition comprising type I IFN and compatible additives,
- possibly a composition comprising a cytokine and compatible additives,
- possibly a composition comprising a cell growth factor and compatible additives,
- possibly a composition comprising at least one antigen, or nucleic acids coding for at least one antigen, to which an immune response is of interest

29. A pharmaceutical composition or a vaccine comprising, as active principle, activated mononuclear APCs according to any one of claims 2 to 4, 24 or 25, together with a pharmaceutically acceptable carrier vehicle or an auxiliary agent, in an amount of about 10^5 to about 10^{10} , and preferably about 10^7 to about 10^8 of said cells per dose administered.

30. A pharmaceutical composition or a vaccine containing, as an adjuvant of an active principle, activated mononuclear APCs according to any one of claims 2 to 4, 24 or 25, in an amount of about 10^5 to about 10^{10} , and preferably about 10^7 to about 10^8 of said cells per dose administered.

31. Use of a cell population according to claims 1 or 25 or of activated mononuclear APCs according to any one of claims 2 to 4 or 24 for the manufacture of a medicament for treating an infectious or neoplastic disease.

ABSTRACT

RAPID GENERATION OF ACTIVATED MONONUCLEAR ANTIGEN PRESENTING CELLS FROM MONOCYTES

The present invention relates to a cell population prepared from blood mononuclear cells, said mononuclear cells being peripheral blood mononuclear cells (PBMC) or CD14+ monocytes, said population containing from about 10 % to about 50 % of activated mononuclear antigen presenting cells (APCs) having the following characteristics:

- they express surface markers CD2, CD83 and CD14, MHC class I and MHC class II molecules
- they secrete TNF- α
- they are able to stimulate allogenic T lymphocytes, as shown by MLR measurements
- they are able to stimulate autologous T lymphocytes proliferation in an antigen specific way.

(no figure)

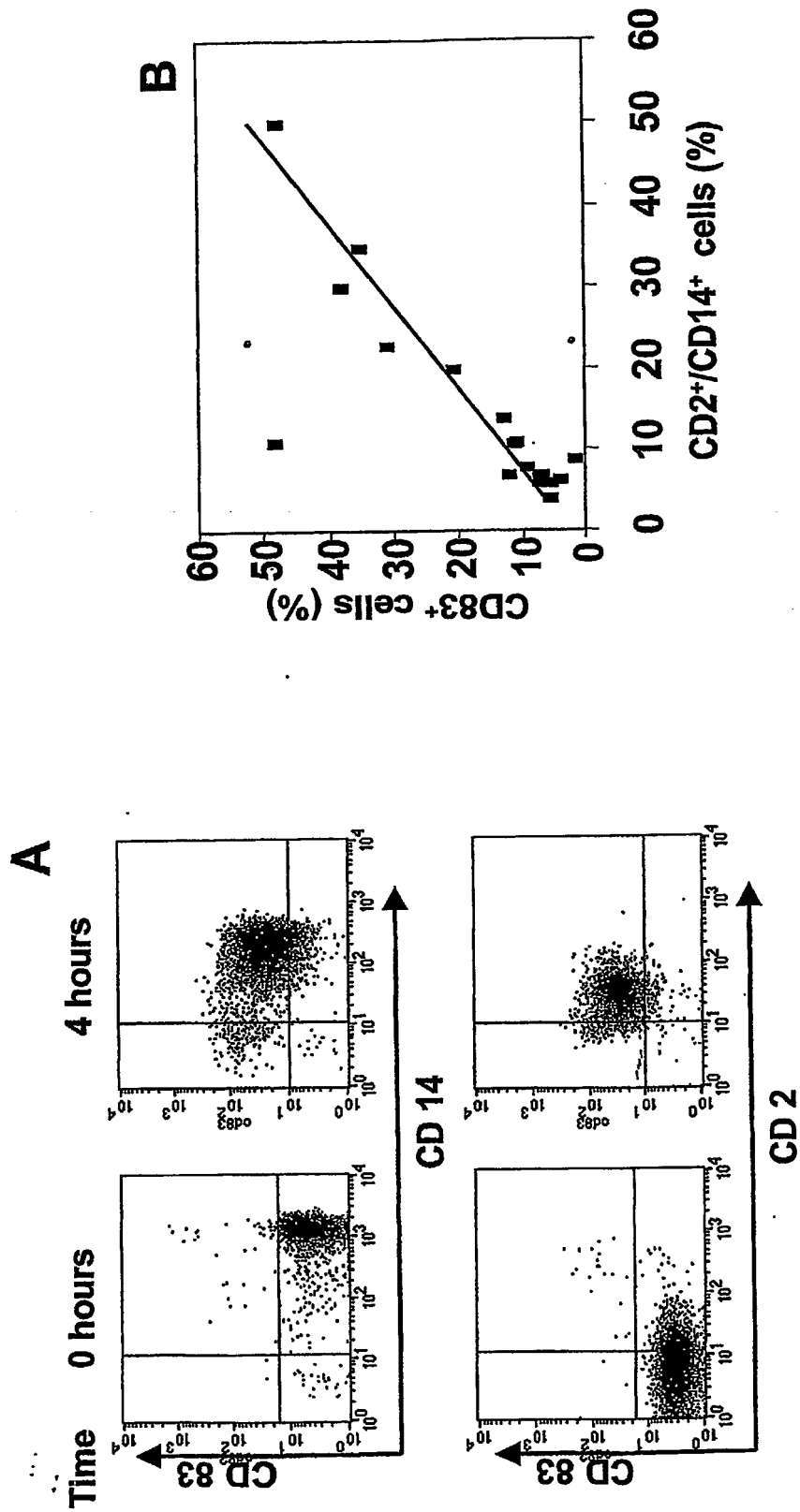
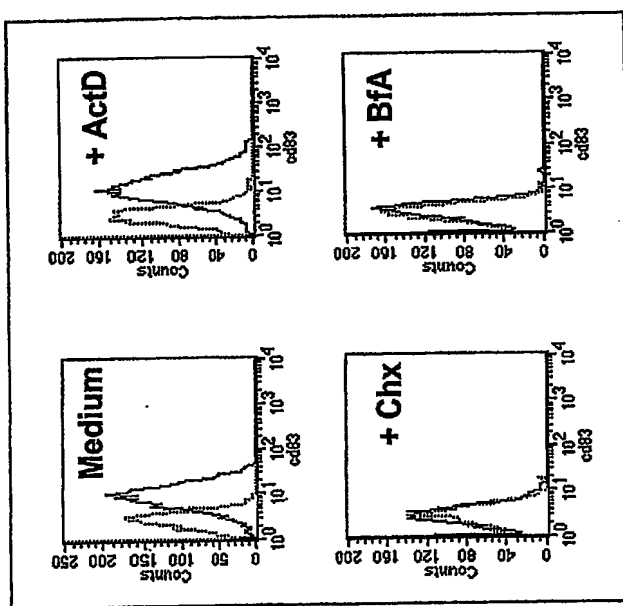
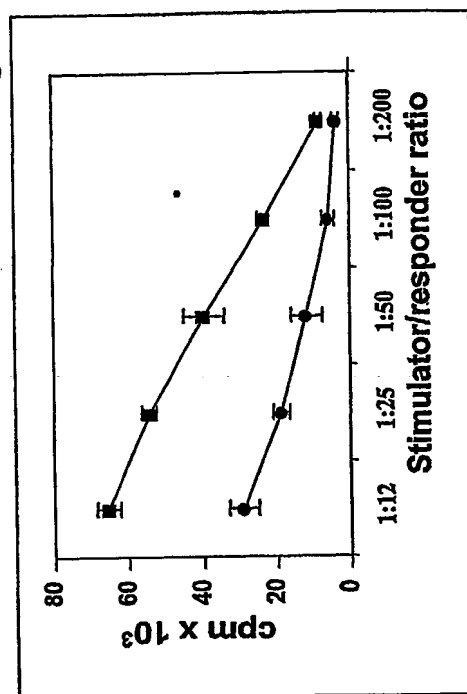
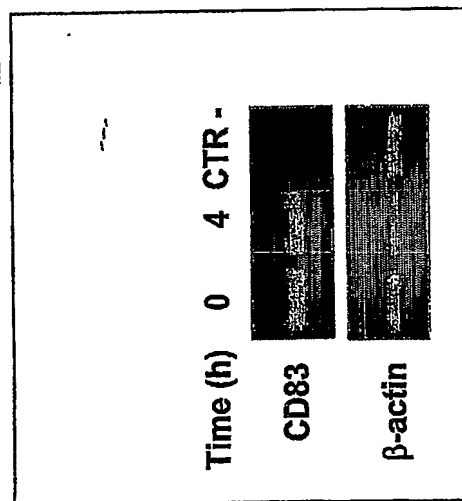


FIGURE 1

A**C****B**

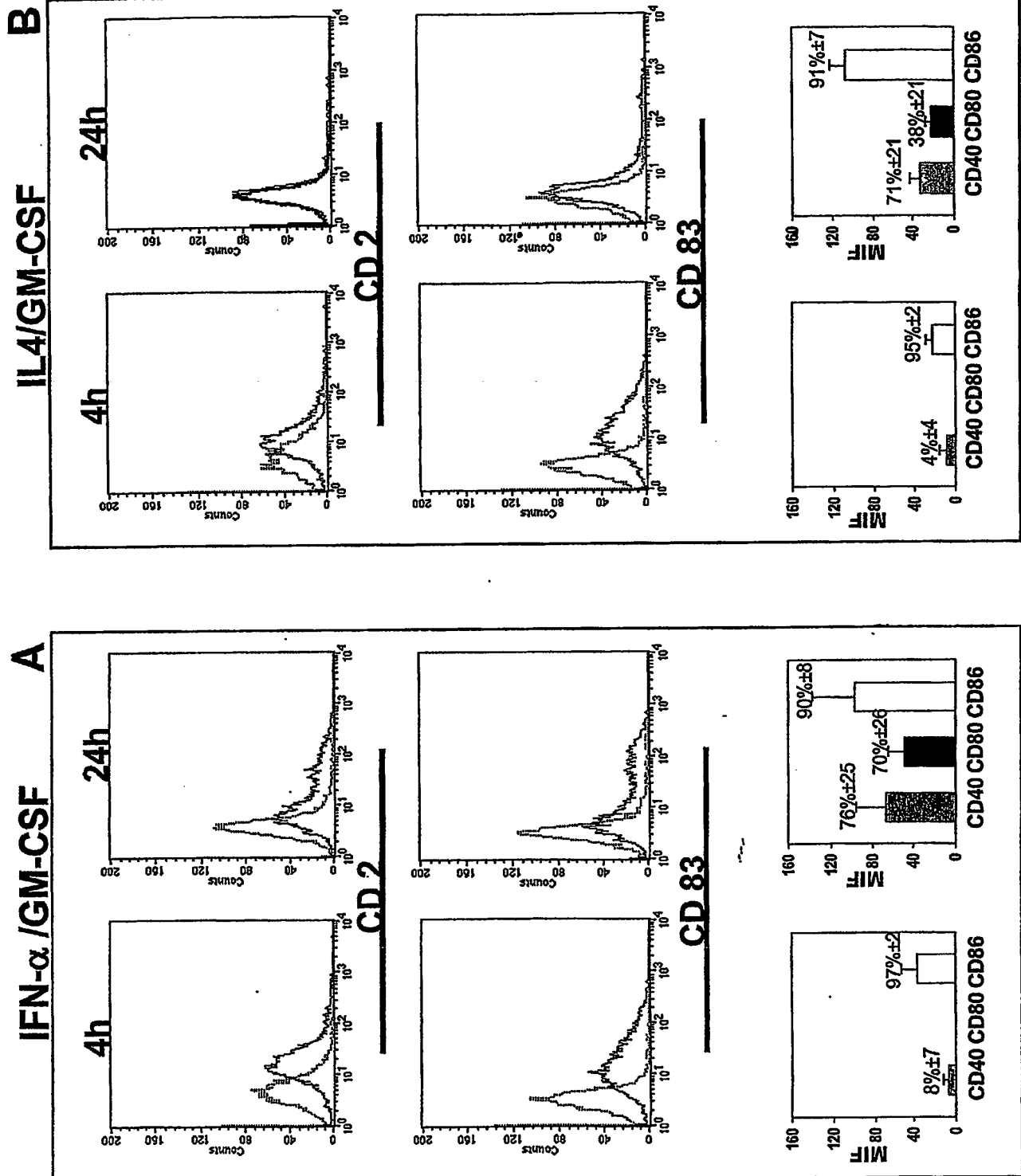
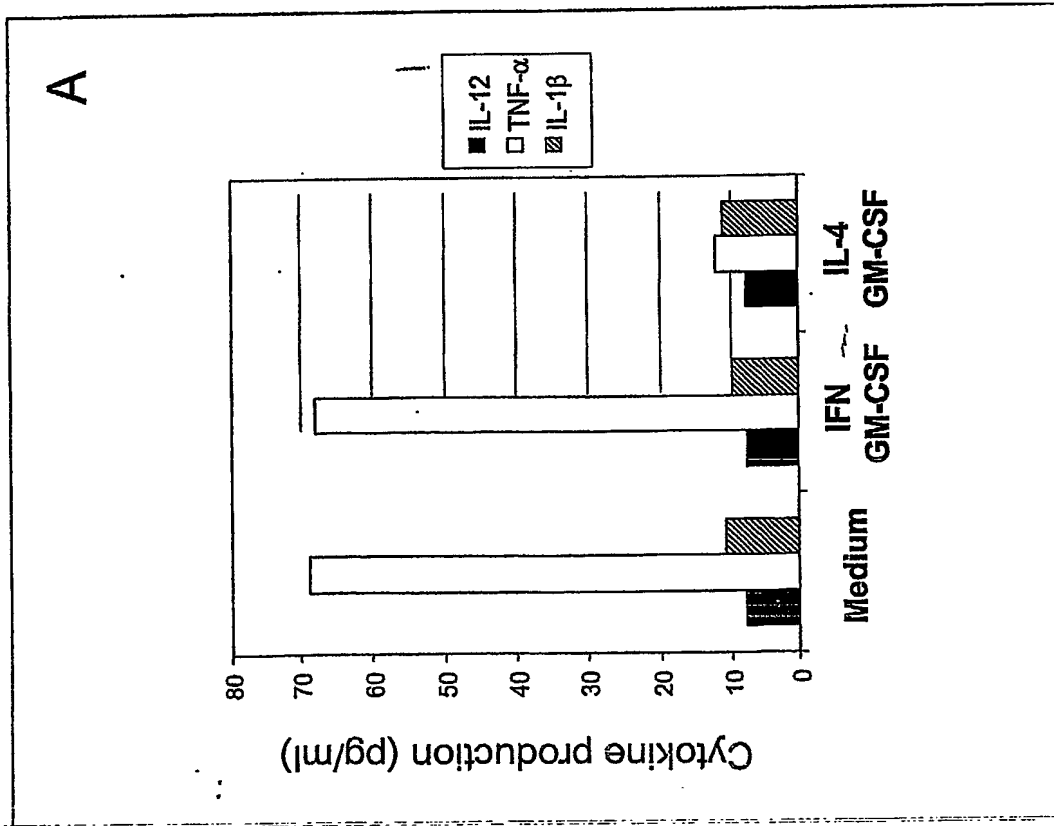
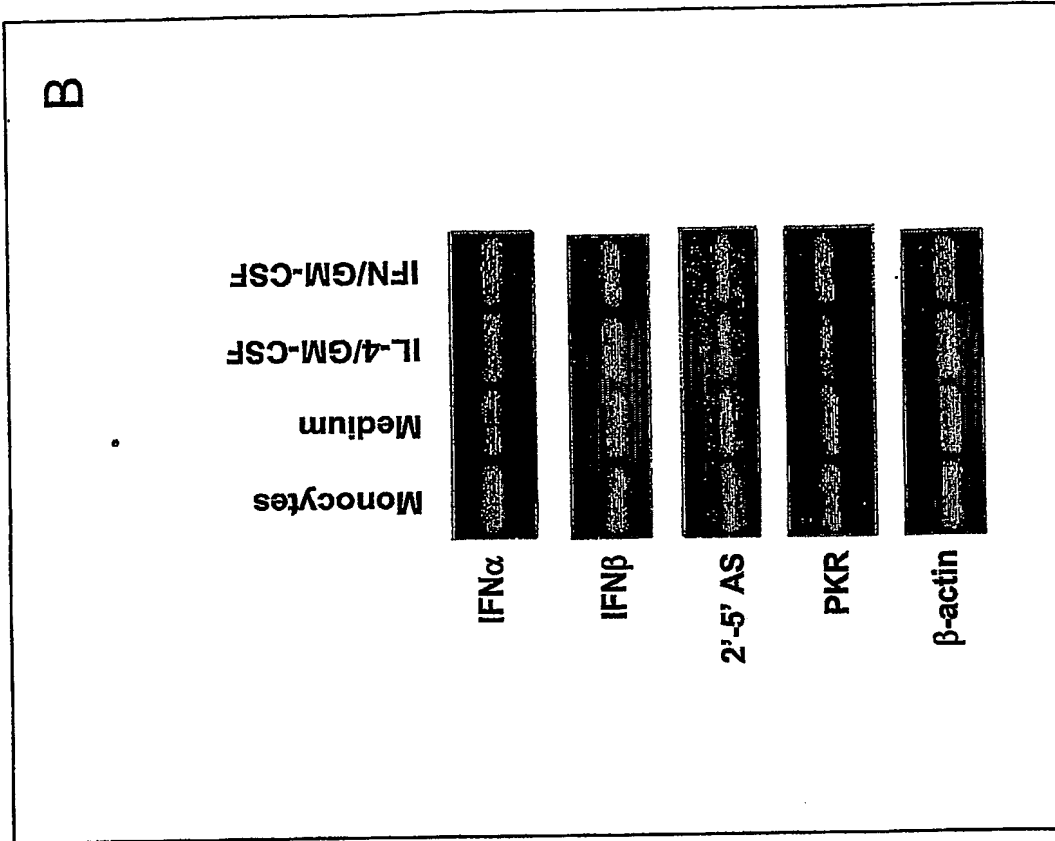


FIGURE 3



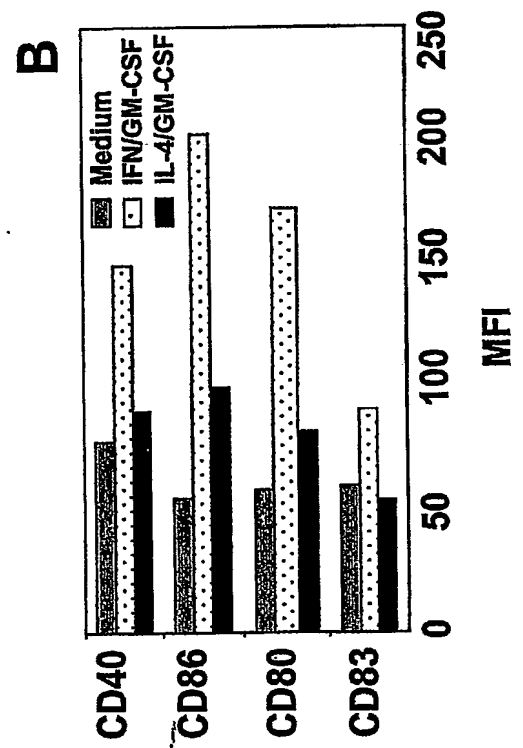
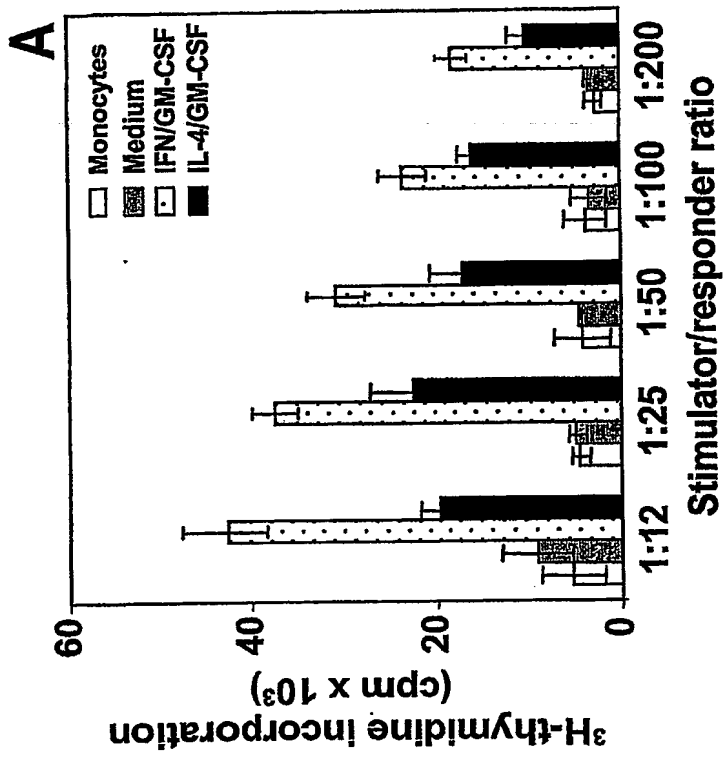
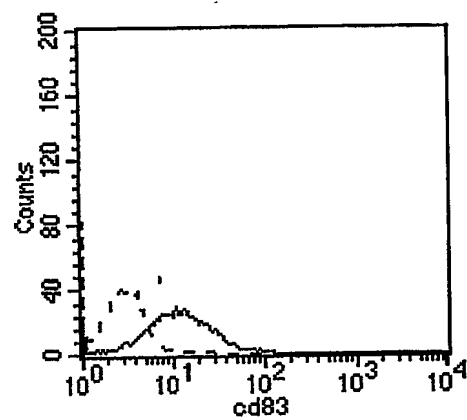
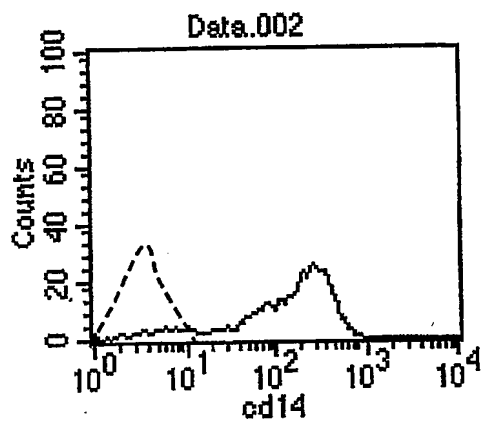
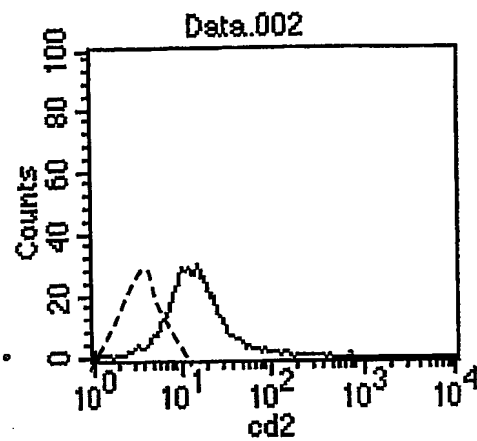
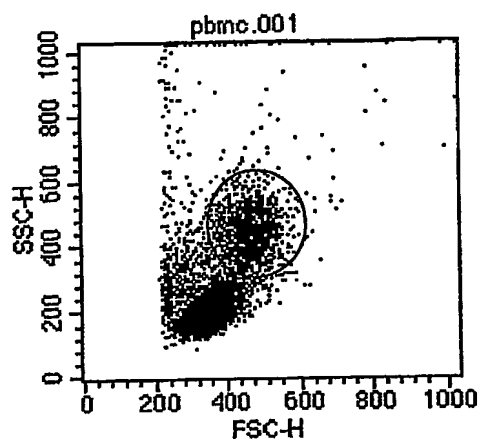


FIGURE 5



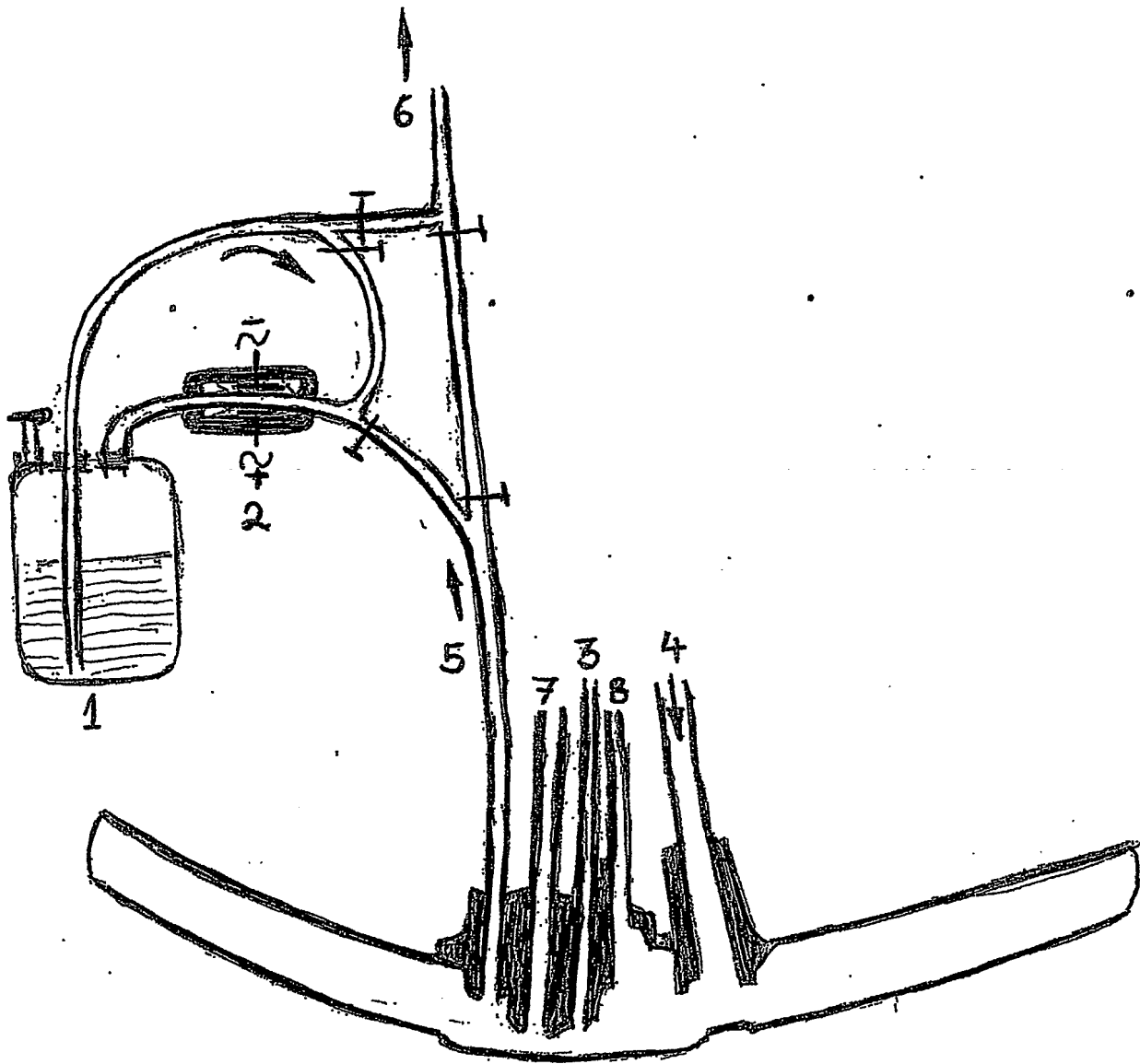


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